Assessment of Workers’ Exposure to Aflatoxin B1 in a Portuguese Waste Industry

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ABSTRACT

Aflatoxin B1 (AFB1) is considered by different International Agencies as a genotoxic and potent hepatocarcinogen. However, despite the fact that the fungi producing this compound are detected in some work environments, AFB1 is rarely monitored in occupational settings. The aim of the present investigation was to assess exposure to AFB1 of workers from one Portuguese waste company located in the outskirt of Lisbon. Occupational exposure assessment to AFB1 was done with a biomarker of internal dose that measures AFB1 in the serum by enzyme-linked immunosorbent assay. Forty-one workers from the waste company were enrolled in this study (26 from sorting; 9 from composting; 6 from incineration). A control group (n = 30) was also considered in order to know the AFB1 background levels for the Portuguese population. All the workers showed detectable levels of AFB1 with values ranging from 2.5 ng ml⁻¹ to 25.9 ng ml⁻¹ with a median value of 9.9 ± 5.4 ng ml⁻¹. All of the controls showed values below the method’s detection limit. Results obtained showed much higher (8-fold higher) values when compared with other Portuguese settings already studied, such as poultry and swine production. Besides this mycotoxin, other mycotoxins are probably present in this occupational setting and this aspect should be taken into consideration for the risk assessment process due to possible synergistic reactions. The data obtained suggests that exposure to AFB1 occurs in a waste management setting and claims attention for the need of appliance of preventive and protective safety measures.

KEYWORDS: aflatoxin B1; occupational exposure; waste management

INTRODUCTION

Aflatoxins are secondary metabolites produced under certain environmental conditions by Aspergillus flavus and Aspergillus parasiticus fungi species (Bhatnagar et al., 2006). These include temperature, water activity, substrate composition, and pH or modified atmospheres (Abdel-Hadi et al., 2012).

Eighteen different types of aflatoxins have so far been identified, of which major members are aflatoxins B1, B2, G1, and G2. Aflatoxin B1 (AFB1) is normally predominant in food cultures and products. This mycotoxin was shown to be genotoxic and a potent hepatocarcinogen (IARC, 1993; Dash et al., 2007). AFB1 is bioactivated by cytochrome P450, a
group of enzymes present more abundantly in the liver and related with the bioactivation and metabolism of several xenobiotics and also endogenous compounds (Josephy, 1997). The CYP450 enzymes bioactivate AFB1 to a unstable metabolite (aflatoxin-8,9-epoxide) that is able to react with cellular macromolecules such as DNA (causing genotoxicity) and proteins (causing cytotoxicity) to form covalent adducts (Autrup et al., 1991; Brera et al., 2002; Doi et al., 2002; Dash et al., 2007; Diaz et al., 2010).

Although dietary exposure to AFB1 has been extensively recognized, evidences suggesting potentially high risks of occupational exposure to AFB1 through inhalation have been accumulating (Dvorackova, 1976; Dvorackova and Pichova, 1986; Baxter et al., 1981; Hayes et al., 1984; Popendorf et al., 1985). Moreover, several epidemiological and laboratory studies have shown that the human respiratory system is also a target for AFB1 carcinogenicity (Hayes et al., 1984; Donnelly et al., 1996; Kelly et al., 1997; Massey et al., 2000).

Workers with exposure to aflatoxins by inhalation, particularly in the form of airborne dust, are prone to ingesting, transmucosally absorbing, and inhaling AFB1 released during tasks involving storing, loading, handling, or milling contaminated materials such as grain, waste, feed, and others (Sorenson et al., 1981; Jargot and Melin, 2013). But there is also the possibility of exposure by dermal absorption which is particularly relevant in workplaces where the use of short clothes is allowed and large skin areas are exposed to particulate matter deposition (Degen, 2008; Mayer et al., 2008).

There are other relevant aspects to consider in the specific case of occupational exposure to mycotoxins, namely: the inexistence of limits to the concentration of airborne mycotoxins and the fact that these compounds are rarely (never in Portugal) monitored in occupational environments (Méheust et al., 2014). Moreover, due to the easiness of fungal detection, fungi are often used as an indirect indicator of mycotoxins presence in occupational settings (Thrane et al., 2004). However, it is important to consider that mycotoxins can be present in the environment long after fungal elimination and not all fungi produce mycotoxins (Halstensen, 2008; Alborch et al., 2011).

The use of biomarkers of internal dose can have an important role in assessing occupational exposure to AFB1 and, consequently, when performing risk assessment. These biomarkers can include metabolites or free aflatoxins in biological samples, including serum plasma, urine, milk, and feces. However, it needs to be taken into consideration that such measurements may provide information not only on aflatoxin intake, but also on the degree of individual absorption (influenced by different factors, such as specific task developed and metabolic rates of each individual) and metabolism itself (Groopman, 1994).

Commonly, waste is disposed by incineration or storage in landfills. Nowadays, aiming to decrease the environmental burden associated with this practice, many European countries have started to apply other treatment methods to avoid and reduce the total amount of waste. As important examples are the separation and collection of organic household waste and, the increase of domestic non-organic waste recycling. However, all these waste treatment methods imply that the workers involved are exposed to different types of risks, namely biological threats (Heldal et al., 2003). Several articles have reported exposure to viruses, bacteria, fungi and their metabolites and, also, to dust (Kiviranta et al., 1999; Wouters et al., 2006; Domingo and Nadal, 2009; Nadal et al., 2009; Park et al., 2011; Schlosser et al., 2011; Duquenne et al., 2013; Viegas et al., 2014a,b). Despite the optimal conditions for fungal growth and, consequently, for mycotoxins production in all the waste management chain, only a few articles were dedicated to study occupational exposure to mycotoxins in this occupational setting (Degen et al., 2003; Mayer et al., 2012).

Considering what has been explained above, the aim of the present investigation was to assess exposure to AFB1 of workers from one Portuguese waste company located in the outskirt of Lisbon.

**MATERIALS AND METHODS**

**Waste company studied**

The waste company enrolled in the study is constituted by different units that are related with the waste management chain. Therefore, there is a sorting unit, a composting unit and an incineration unit. The waste sorting unit (52 workers) has a maximum capacity of 90 500 ton year$^{-1}$ of urban waste and works 5 days a week. The composting unit has a maximum capacity of 40 000 ton year$^{-1}$ of organic waste and also works
5 days a week (30 workers). Finally, the incineration unit has a maximum capacity of 662 000 ton year$^{-1}$ and works every day of the week (71 workers).

**Detection of AFB1 in serum of waste workers**

Occupational exposure assessment to AFB1 was done with a biomarker of internal dose that measures AFB1 in the serum. The principal objective was to obtain data regarding recent exposure to AFB1 and also its level of intensity. This approach is useful for rapid screening of samples for acute exposures but also reflects chronic exposure.

Forty-one workers from the waste company were enrolled in this study (26 from sorting; 9 from composting; 6 from incineration). The samples collection was done in different days from the last 6 months of 2013. A control group ($n = 30$) was also considered in order to know the AFB1 background levels for the Portuguese population. This group was composed of subjects who conducted administrative tasks in an educational institution without any type of activity known to involve exposure to AFB1. All participants signed a consent form and were provided with the study protocol. The same approach was followed in Viegas et al. (2012, 2013a,b) and is recommended by other authors (Mayer et al., 2003; Degen, 2011).

Additionally, the workers answered a questionnaire that contained questions on personal data, such as age, detailed current and previous occupational history, tasks developed in the two days before related with waste management, activities developed outside the company (such as agriculture or animal production). The questionnaire was filled during a personal interview.

Although workers and control group are originated from the same geographical zone and have a similar age distribution there might be some differences in the diet related with different genera distribution and, also, social and educational background. Unfortunately, it was not possible to study those differences which can have some influence in AFB1 levels.

**Blood sample preparation**

All blood samples were subjected to centrifugation to obtain serum, subsequently stored at $-20^\circ$C until further analysis. Five hundred microliters of serum was incubated for 18 h at 37$^\circ$C with pronase (Calbiochem, 50 U per 5 mg protein) before application to pre-wet C18 column (RIDA C18 column, R-Biopharm). The column was washed with 5 ml 5% methanol to remove small peptides and amino acids. The fraction containing aflatoxin was eluted with 80% methanol, which was posteriorly evaporated under a nitrogen stream and diluted to reach a 10% methanol solution. The eluate was then applied to an immunoaffinity aflatoxin column (Easi-Extract Aflatoxin; R-Biopharm) and the aflatoxin-containing fraction was eluted with 1 ml methanol in phosphate buffer 0.1 M, pH 7.4 (1:1), after rinsing the column with phosphate-buffered saline.

**ELISA assay**

For AFB1 quantification, the RIDASCREEN AFB1 30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) was used, and was calibrated with aflatoxin standards from 1 to 50 ng ml$^{-1}$. Values below 1 ng ml$^{-1}$ were considered nondetectable since these are below the detection limit. Samples or standards were pipetted into the wells already coated with capture antibodies directed against anti-aflatoxin. Prior to the addition of AFB1-antibody solution, AFB–enzyme conjugate was added. After 30 min of incubation, the wells were washed three times. Indicator color was obtained by adding a substrate/chromogenic solution to each well and the reaction was stopped after 15 min with a termination solution. Absorbance was measured at 450 nm and results were assessed with Ridasolf Win software version 1.73 (R Biopharm).

**Statistical analysis**

The Mann–Whitney test was applied to compare the two groups under study: waste workers and controls. SPSS (Statistical Package for Social Sciences) 21.0 was used to perform all the statistical analysis. To compare the AFB1 within the three sampling locations (sorting, composting, and incineration) the Kruskal–Wallis test was used.

**RESULTS**

Blood samples were collected from a total of 41 workers and 30 controls. Characteristics of these groups are summarized in Table 1.

All the workers showed detectable levels of AFB1 with values ranging from 2.5 to 25.9 ng ml$^{-1}$ and with a median value of 9.9 ± 5.4 ng ml$^{-1}$ (Table 2).
The higher value (25.9 ng ml\(^{-1}\)) was obtained in a woman from the sorting unit and the lower value (2.5 ng ml\(^{-1}\)) was obtained in a man from the incineration unit (maintenance service). No significant differences were detected between workers of different gender. Moreover, six workers (14.6%) obtained results higher than 20 ng ml\(^{-1}\) and the rest obtained values below 14 ng ml\(^{-1}\). These six workers had the following distribution: four are from the sorting unit and two are from the composting unit.

In the control group, the AFB1 values were all below 1 ng ml\(^{-1}\). Since the AFB1 result is not a pure quantitative variable, it is considered an ordinal variable, which is the basis for using the nonparametric Mann–Whitney test to compare concentrations of AFB1 between the two groups. When the concentration was less than 1 ng ml\(^{-1}\) (limit of detection [LOD]), this was considered nondetectable.

Significantly higher concentrations of mycotoxin were found in waste workers compared to controls. However, between workers from the different units (sorting, incineration, and composting) we did not observe any statistically significant difference \((\chi^2_{(KW,2)} = 2.657, P = 0.265, \text{median sorting} = 10.12 \text{ ng ml}^{-1}, \text{median composting} = 10.40 \text{ ng ml}^{-1}, \text{median incineration} = 9.24 \text{ ng ml}^{-1})\).

**DISCUSSION**

This is the first study developed in Portugal aiming to assess occupational exposure to mycotoxins in a waste management setting. Contrary to fungi, exposure to mycotoxins is not usually identified as a risk factor in this (and other) occupational setting. However, it seemed particularly important to assess exposure to a mycotoxin that is classified as a carcinogenic agent, such as AFB1 (IARC, 1993, 2002). This was also supported by the fact that, in this setting, recent published work presented fungi contamination data related with species that are recognized as AFB1 producers (Viegas et al., 2014a, b). However, although fungi information can provide a rough estimate for mycotoxin presence, it is always better to confirm exposure and perform risk assessment by studying directly the mycotoxins presence in the environment or in Humans (Jargot and Melin, 2013). That was the intention of the present research work: to assess the real exposure to AFB1 of workers from a waste management industry.

The data obtained here leads to the conclusion that occupational exposure to AFB1 is occurring in waste management setting. This is supported by the fact that every worker enrolled in this research presented measurable levels of AFB1 unlike controls, which did not present AFB1 in their serum (below the LOD). With these results it is also possible to conclude that the workplace is the single factor contributing to the exposure to this carcinogenic agent. This is particularly important because a possible causative relation between occupational exposure to AFB1 and cancer has already been presented in different occupational settings (McLaughlin et al., 1987; Olsen et al., 1988; Autrup et al., 1993).

Similar research work has shown the same results and also highlighted the importance for the simultaneous contamination by particulate matter of the work environments (Viegas et al., 2012, 2013a, b). The effect
of dust as a carrier of AFB1 to the breathing zone and mouth has been previously discussed (Autrup et al., 1991; Brera et al., 2002; Jargot and Melin, 2013). There is evidence from several studies that the contamination by particulate matter in the waste management setting is frequent and intense (Malmros et al., 1992; Tolvanen et al., 2005; Tolvanen and Hänninen, 2007; Domingo and Nadal, 2009; Viegas et al., 2014b; Viegas et al. 2014). Therefore, particulate matter is probably contributing significantly for the exposure to this mycotoxin. Moreover, in the units studied, the use of respiratory protection devices was not mandatory and was common to observe workers without this kind of protection in their workplaces.

Furthermore, several recent published papers stated that there is metabolic activation of AFB1 in the lung even when dealing with low environment concentrations (Yang et al., 2012, 2013; Zhang et al., 2014). Therefore, the lung can also be a target for AFB1 carcinogenicity and, considering our data, this can be a real risk to bear in mind in the waste management setting.

The importance of dermal contact for the total AFB1 internal dose is still unclear as it is impossible to estimate what is the real contribution of this exposure route. Previous studies have shown that skin is permeable to AFB1 and other mycotoxins (Kemppainen et al., 1988; Boonen et al., 2012). This exposure route can have a significant role if the workers are sweating due to tasks involving high metabolic rates or if they use short clothes. In the working units under study, the workers were usually wearing t-shirts and not always had gloves and, particularly in the sorting unit, the manual work is very intense involving probably high metabolic rates. It is likely that these two aspects could contribute to explain the higher levels of AFB1 obtained in workers from the sorting unit (25.9 ng ml\(^{-1}\)).

This occupational setting is characterized by having more male than female workers as it was observed in our workers’ population. Differences in the results between genera were not found probably due to the fact that five of the six females work in the manual sorting, where there is a direct and manual contact with the waste. Moreover, in the group of higher exposure, two of the six workers were female. One of them was working in the waste sorting, directly involved in the manual sorting of the waste and the other in the laboratory, where the waste sample analyses takes place.

In most of the studies already developed, exposure to AFB1 was assessed by detecting its presence in the occupational environment (air, settled dust, material that is being handled) (Mayer et al., 2012; Selim et al., 1998). In this research work, AFB1 exposure was assessed by the use of a biomarker that reflects the total absorbed dose. Both data (external dose and internal dose) are important and relevant for the risk assessment process and environmental and biological monitoring should be carried out in workplaces where possible contaminated material is handled (Iavicoli et al., 2002). This complementary information prompts us to identify possible exposure sources. However, it is possible to conclude that biomarker data is a more accurate reflection of exposure because it measures the real quantity of AFB1 in the organism (Marin et al., 2013). Indeed it bears in consideration all the individuals and all the task differences that can influence the concentration of airborne mycotoxins and absorption rates such as the use of protection devices, ventilation resources or high metabolic rates needed to perform a specific task. Still, it is important to note that the biomarker used does not allow the assessment of the cancer risk for this occupational population. For this goal, AFB1–DNA adducts is a more suitable biomarker which was first used in a nested case–control study, in which urinary aflatoxin adducts (aflatoxin N7-guanine) were found to be significantly associated with subsequent development of liver cancer in Chinese men (Qian et al., 1994, Pottenger et al., 2014). Since then other studies selected this biomarker as a good predictor of cancer risk due to exposure to AFB1. Importantly, other biomarkers should be used when assessing cancer risk. A specific example is the detection of polymorphisms in genes that have an important role in AFB1 metabolism (Groopman et al., 1993; Pottenger et al., 2014).

In what refers to our results, it is important to consider a possible underestimation of exposure due to the fact that only AFB1 concentration was measured and not their metabolites, that can also be present due to, as mentioned before, lung metabolizing action (Viegas et al., 2012).

Our results showed much higher AFB1 (8-fold higher) values in workers from a waste plant than in workers from other Portuguese settings studied, including poultry production (Viegas et al., 2012) and swine production (Viegas et al., 2013a). Only a study
developed in Nigeria (Oluwafemi et al., 2012) that also intended to analyze the occupational exposure to AFB1 in workers from a feed mill showed higher values than the ones present in our study (mean value = 189.2 ng ml⁻¹ vs mean value = 11.19 ng ml⁻¹). The results obtained are probably due to three important factors: the continuous fungal contamination that occurs during all the waste management chain resulting in a continuous waste mycotoxin contamination; the fact that mycotoxins are resistant to adverse environment factors such as high or low temperatures and can be present in the environment or waste long after death and disintegration of the species producing it (Halstensen, 2008; Alborch et al., 2011); and, finally the fact that in the waste management setting, workers are repeatedly exposed to fungi during the whole work shift, situation that does not occur in the poultry or swine production where workers are often outside the pavilions.

These three factors combined with the inexistence of legislation and exposure limit (as in other settings, such as food and animal feed), and the huge amount of waste that is handled in each of the studied unit’s results in high exposure to airborne mycotoxin every time the workers have to handle or be near to the waste. This last aspect is well demonstrated by the fact of the six workers with higher AFB1 levels are coming from sorting and composting units, where activities developed implicate waste handling or higher proximity to the waste.

As mentioned before, AFB1 is not volatile and uses probably the particulate matter to reach the respiratory system of the workers. Considering this, mycotoxin exposure will be most intensive in tasks that involve high exposure to particulate matter. This fact explains the differences obtained in our results: Only six workers (14.6%) had AFB1 levels higher than 20 ng ml⁻¹, while the rest of the workers obtained values below 14 ng ml⁻¹. These six workers were involved in tasks that imply high release of dust such as manual sorting of waste and driving waste removing vehicles. There are other tasks associated with high exposure to particles, namely cleaning activities with the use of brooms or compressed air. Considering all these aspects it is possible to estimate that avoiding exposure to particles will minimize exposure to fungi and to mycotoxins. Some preventive measures can be pointed out, namely the use of vacuum cleaners with adequate dust retention, information and education of the workers to the use of adequate personal protective devices (respiratory and dermal devices) and to wash the skin exposed during dusty tasks.

Since this work was based in just a single moment of sampling, it is not possible to know whether the values found here are normally present on the workers or if they were related with a specific condition of their work environment or a specific task developed on the last days. Regarding this, it is important to promote future research aiming at investigating whether mycotoxin exposure differs in different waste and workplace environment conditions and, also, seasons. Nevertheless, it is important to note that the occasional exposures to high concentrations (peak) is of concern as it probably implicates a different biologic response when compared with the response obtained upon exposure by food ingestion (regular exposure to low concentrations), the most and better studied exposure scenario. Therefore, the peak exposures can result in different and more dramatic health effects (Smith, 2001; Preller et al., 2004).

In waste management setting it is also important to take into account a potential simultaneous exposure to more than one mycotoxin and the possible chemical interactions between mycotoxins and other hazardous compounds, like endotoxins and particles with different chemical composition (Park et al., 2011; Mayer et al., 2012). Indeed, in addition to A. flavus, other fungal species recognized as mycotoxin producer, were found in the same plants (Viegas et al., 2014a,b). For instance, in the sorting and composting units the most prevalent fungi found in air were species from the complexes Aspergillus niger, A. flavus and Aspergillus fumigatus, and in the incineration unit, besides species from A. fumigatus and A. flavus complexes, also Penicillium genera was present between the most prevalent (Viegas et al., 2014). Considering fungal characterization in these waste management units, we must take in account also multiple exposures to mycotoxins from the Aspergillus genera, such as gliotoxin. In a study developed in 2005, this mycotoxin was detected in 93% of A. fumigatus cultures recovered from patients at a Tertiary Care Cancer Center (Lewis et al., 2005). In this study, ochratoxin A was also detected, since among A. niger complex, several species are producers of this mycotoxin as well (Samson et al., 2004). Additionally, in a previous study developed by Degen...
et al. (2003) ochratoxin A was also found in blood samples from workers at waste handling facilities. Other study developed recently by Mayer et al. (2012) in waste recycling plants found 33 mycotoxins and 5 bacterial metabolites in settled dust. This particular work explains very well the challenge associate with all the possible interactions between metabolites produced by different species. As expected and described already in other studies published (Speijers and Speijers, 2004; Klarić, 2012; Klarić et al., 2013), the toxicokinetic and toxicodynamic aspects are changed when two or more mycotoxins are present. In practice, the consequence of combined exposure to mycotoxins can either quantitatively or even qualitatively be different from what would be predictable when considering only one isolated mycotoxin (Speijers and Speijers, 2004, Klarić et al., 2013). Therefore, the effects of possible interactions need to be considered in the risk assessment process, probably meaning that lower doses can have the same or worst health effects (Sexton and Hattis, 2007).

Considering all the above and, in addition to the implementation of preventive measures, it is important that the workers of this occupational setting have an adequate health surveillance program. This program could involve for example the use of specific biomarkers for hepatic tumors. These have previously been proposed to be applied on occupational health interventions (Saad-Hussein et al., 2013). Although with low specificity, they can be considered the first sign of health effects due to exposure to hepatic carcinogens.

CONCLUSIONS
The results obtained in this work suggest that exposure to AFB1 occurs in waste management setting and may be related with the high contamination of waste being handled. Preventive and protective measures need to be developed to avoid exposure and the health effects associated with this carcinogenic agent.

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REFERENCES


